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SOIL REACTION AND THE GROWTH OF AZOTOBACTER

[PRELIMINARY PAPER]

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INTRODUCTION

It has frequently been observed in this and other laboratories that, when soils are examined for Azotobacter, some give, on a mannite nutrient solution, a characteristic dark-brown film composed almost wholly of Azotobacter cells. Others give no visible surface growth. During the summer of 1917 a preliminary survey was conducted to ascertain to what extent soils in the vicinity of this Station exhibited the above variations. In all, 90 soils were collected within a radius of 2 miles of the laboratory. These samples were taken from as widely varying conditions as could be located. Some were collected from the highest hills and others from the lowest overflow bottom land, one even from a sand bar in the Kansas River. Samples of soil were taken from all of the following soil conditions: Cultivated, permanent alfalfa, pasture, roadsides, hedges, river and creek banks, and forests. Some of the spots from which samples were obtained were very fertile, while others were practically barren.

EXPERIMENTAL WORK

In collecting the soil for examination the ordinary precautionary methods used to prevent contamination were observed. The soil sample examined was a well-mixed composite of six or more smaller samples collected within a few yards of each other. When convenient, soil was taken to a depth of approximately 6 inches. As soon as possible the samples were brought to the laboratory, and sterile Erlenmeyer flasks containing 50 cc. of the following cultural solution were immediately inoculated. The composition of the cultural solution was: Di-potassium phosphate (K_2HPO_4), 0.2 gm., magnesium sulphate ($MgSO_4$), 0.2 gm., sodium chlorid (NaCl), 0.5 gm., mannite, 20 gm., ferric chlorid ($FeCl_3$), trace, distilled water, 1,000 gm. After all the salts had been dissolved, the solution was rendered slightly alkaline to phenolphthalein with sodium

hydroxid. Flasks were inoculated in quadruplicate with 10 cc. of a suspension made by shaking 100 gm. of soil in 200 cc. of sterile water. Two of the flasks were immediately sterilized, and all were incubated at room temperature for three weeks. The remaining soil was spread out in a thin layer, allowed to dry thoroughly, and stored for future physical and chemical study.

During incubation the growth was observed at frequent intervals, and microscopic examinations of the surface growth were made both at the end of one and at the end of three weeks. After incubation total-nitrogen determinations were made on all samples, and that present in the sterilized controls was deducted from that in the cultures. In all except a very few instances the growth in duplicate cultures was similar both macroscopically and microscopically. The quantity of nitrogen present in duplicates also checked within very narrow limits except in a few instances.

EXPERIMENTAL RESULTS

In Table I are given the sample number, date of collection, soil type, condition of ground, type of growth observed, average nitrogen fixed per culture, expressed in milligrams, and the reaction of the soil, expressed in P_H.

Under "Type of growth" the terms "typical," nontypical," and "none" have been used. Those designated as "typical" conform quite well with previously described cultures of *Azotobacter chroococcum*. The growth was a uniform brown to black film covering the entire surface and composed almost entirely of Azotobacter cells. The "nontypical" samples exhibited usually a heavy, more or less gelatinous, irregular, gray, yellowish, or even an irregularly brown spotted film. Under the microscope such a film was found to be composed of numerous types of bacteria, fungi, and protozoa. Always, however, there were large numbers of organisms similar to, if not identical with, Azotobacter. Those cultures designated as "none" gave very little, if any, surface growth and Azotobacter-like cells were never observed. In some instances such cultures exhibited a copious gas formation, while in others there was no visible evidence of growth. In most, if not all, cultures butyric acid was formed; especially was this true where gas formation took place.

In 37 samples, or 41 per cent, no Azotobacter developed. The nitrogen fixed in such cultures varied from −0.66 to 5.55 mgm. per culture, with an average of 3.88 mgm. In 28 samples, or 31 per cent, the growth was nontypical. The nitrogen fixed in these cultures varied from 3.41 to 9.95 mgm., with an average of 7.09 mgm. per culture. In 25 samples, or 28 per cent, the typical growth occurred. The quantity of nitrogen fixed in these cultures varied from 7.95 to 10.95 mgm., with an average per culture of 9.47 mgm.

TABLE I.—*Relation between soil type, condition of soil, growth of Azotobacter, nitrogen fixed per 50 cc. of culture, and reaction of soil solution*

Soil No.	Date.	Soil type.[a]	Condition of ground.	Type of growth.	Nitrogen fixed.	Reaction expressed as P_H.
					Mgm.	
1	Apr. 19	Wabash silt loam	Cultivated	Typical	10.34	6.9
2	do	Colluvial Marshall silt loam	Forest	None	1.98	5.4
3	do	Marshall silt loam	Cultivated	do	2.98	5.6
4	do	Oswego silt loam	Sod	Nontypical	6.76	6.9
5	do	Marshall silt loam	Alfalfa	do	5.39	7.1
6	do	do	Cultivated	None	4.40	5.7
7	Apr. 23	do	Alfalfa	do	4.35	5.7
8	do	do	do	do	4.62	5.6
9	do	do	do	do	4.67	5.5
10	do	do	do	do	—.60	5.6
11	do	do	do	do	1.65	5.8
12	do	do	do	do	4.52	5.9
13	May 8	do	Cultivated	do	3.19	5.6
14	do	Oswego silt loam	Forest	Typical	8.52	7.4
15	do	do	do	do	10.28	7.4
16	do	Summit Silt loam	Cultivated	None	4.02	5.6
17	do	do	Pasture sod	do	3.02	5.3
18	do	Marshall silt clay loam	Cultivated	do	3.74	5.6
19	May 12	do	do	do	4.51	5.7
20	do	do	do	do	4.73	5.4
21	do	do	do	do	4.68	5.6
22	do	do	do	do	4.73	5.6
23	do	do	do	do	4.68	5.6
24	do	Oswego silt loam	Alfalfa	do	4.18	5.6
25	May 13	Wabash silt clay loam	Cultivated	Nontypical	6.38	7.0
26	do	do	Alfalfa	Typical	9.90	6.6
27	do	do	Cultivated	Nontypical	7.54	6.1
28	do	do	do	Typical	9.52	6.2
29	do	Marshall silt clay loam	Brook bottom	Nontypical	8.80	7.6
30	do	Wabash silt loam	Pasture sod	None	4.07	5.6
31	May 23	Laurel silt loam	Cultivated	Typical	10.62	7.5
32	do	Colluvial Summit silt loam	Alfalfa	None	5.55	5.9
33	do	do	Orchard	do	3.96	6.7
34	do	Summit silt loam	Alfalfa	do	4.51	6.8
35	do	Stony loam	Pasture sod	Nontypical	6.76	7.6
36	do	(?) Silt loam	Cultivated	do	9.18	6.0
37	May 25	Wabash clay loam	do	None	5.44	6.2
38	do	Wabash silt clay loam	do	Nontypical	3.90	5.6
39	do	do	Orchard	do	8.38	6.1
40	do	Summit silt loam	Cultivated	do	8.14	7.0
41	do	Marshall silt loam	Alfalfa	do	7.59	6.0
42	do	Wabash silt loam	Cultivated	do	8.25	7.4
43	May 31	Laurel silt clay loam	do	Typical	9.62	7.7
44	do	Laurel very fine sandy loam	do	Nontypical	9.95	7.5
45	do	do	Orchard	Typical	10.12	7.4
46	do	Laurel fine sandy loam	Sod	None	1.04	5.9
47	do	Laurel very fine sandy loam	Hedge	Typical	10.01	7.4
48	do	do	Roadside	Nontypical	6.65	6.4
49	June 8	Marshall clay loam	Cultivated	None	2.75	5.5
50	do	Marshall silt loam	Alfalfa	do	3.96	5.8
51	do	do	Pasture sod	do	3.24	5.3
52	do	Summit silt loam	Forest	Nontypical	5.50	7.3
53	do	Marshall silt loam	do	Typical	9.02	7.7
54	do	Colluvial Summit silt loam	Orchard	Nontypical	7.42	6.0
55	June 12	do	Cultivated	Typical	8.69	7.5
56	do	Summit silt loam	Alfalfa	Nontypical	7.15	7.4
57	do	Oswego silt loam	Cultivated	do	7.59	7.5
58	do	Summit stony loam	Pasture sod	None	3.96	5.5
59	do	Summit silt loam	Cultivated	Typical	10.62	7.4
60	do	Oswego silt loam	Ravine	None	4.56	5.8
61	June 15	Wabash silt clay loam	Cultivated	do	3.68	5.5
62	do	(?) silt loam	Forest	Typical	7.26	7.5
63	do	Summit silt loam	Cultivated	do	10.95	7.4
64	do	do	do	do	8.30	6.1
65	do	Marshall silt loam	Orchard	None	5.17	5.7
66	do	do	Alfalfa	do	3.85	5.7
67	June 26	Wabash silt loam	Cultivated	do	4.07	5.5
68	do	Oswego silt loam	do	Nontypical	6.88	6.1
69	do	Marshall silt loam	do	None	4.12	5.6
70	do	do	Alfalfa	Typical	8.69	6.8
71	do	Wabash silt loam	Cultivated	Nontypical	6.87	5.6
72	do	Wabash silt clay loam	Pasture	do	6.76	7.0
73	June 28	Osage silt loam	Creek bank	None	4.51	5.9
74	do	do	Cultivated	Nontypical	8.08	7.4

[a] The writer is indebted to Prof. R. I. Throckmorton, of the Department of Agronomy, for the classification of the soils. Where the question mark (?) is used, it was impossible to identify the type with accuracy.

TABLE I.—*Relation between soil type, condition of soil, growth of Azotobacter, nitrogen fixed per 50 cc. of culture, and reaction of soil solution*—Continued

Soil No.	Date.	Soil type.	Condition of ground.	Type of growth.	Nitrogen fixed.	Reaction expressed as P_H.
					Mgm.	
75	June 28	Osage silt loam	Cultivated	Nontypical	6.08	7.5
76	do	do	do	do	3.41	5.5
77	do	do	Alfalfa	None	4.20	5.6
78	do	Summit stony loam	Forest	Nontypical	5.50	7.7
79	June 30	Laurel medium sand	Sand bar	do	7.59	7.7
80	do	Laurel very fine sandy loam	River bank	do	9.24	7.6
81	do	Laurel fine sandy loam	Cultivated	Typical	9.62	7.4
82	do	(?)	Forest	do	7.97	7.7
83	do	Colluvial Summit silt loam	Cultivated	do	9.90	7.6
84	do	Laurel fine sandy loam	Weedgrowth	do	9.51	7.5
85	July 11	Osage fine sandy loam	Cultivated	do	(a)	7.5
86	do	Osage silt loam	do	Nontypical	(a)	7.5
87	do	Summit stony loam	Creek bank	Typical	(a)	7.8
88	do	Osage silt loam	Alfalfa	do	(a)	6.9
89	do	Osage fine sandy loam	Creek bank	do	(a)	7.5
90	do	Osage silt loam	Cultivated	do	(a)	7.3

a Quantitative nitrogen determination was not made.

DISCUSSION OF RESULTS

In order to show that the observed differences in Azotobacter growth and nitrogen fixation were not due to faulty technic, a soil known to possess a high nitrogen-fixing power was cultured in parallel as a control. In every instance this soil gave a typical film. The nitrogen fixed varied from 10.12 to 12.05 mgm., with an average of 10.50 mgm. per culture. There is evidently, therefore, a wide variation in the nitrogen-fixing power and in the Azotobacter development from the soils examined.

Efforts to correlate this variation with soil type, moisture content of soils, condition of soil with respect to cultivation, fertility, etc., gave negative results. In some instances soils of a similar type and collected very close to one another gave, on the one hand, good growth and nitrogen fixation, and, on the other, no surface growth. Many soils in high state of fertility gave no Azotobacter, while other almost barren or noncultivated soils gave excellent growth and high nitrogen fixation.

The only gross factor that the presence or absence of Azotobacter could in any way be associated with was the elevation from which the samples were taken. As a rule those soils coming from the higher elevations gave no Azotobacter growth, while those from the lower levels gave growth. There were, however, a number of marked exceptions to these rules. For example, soil 35 was from the top of a barren hill, while 37 was from low bottom land; No. 35 gave Azotobacter growth, while No. 37 did not.

The presence of Azotobacter in soils has frequently been associated both with the presence of calcium carbonate and with the reaction. From available evidence there seems to be no doubt that soils well supplied with calcium carbonate and necessarily alkaline give in cultural

solutions a more vigorous development of Azotobacter than do soils deficient in lime. There are, however, too many exceptions to this rule to regard the presence or absence of calcium carbonate alone as the controlling factor The available evidence regarding the influence of soil reaction upon the presence therein of Azotobacter has been obtained by methods which permit such wide discrepancy in results that they can be regarded only as indicative and not conclusive.

Christensen[1] has carried out by far the most carefully executed experiments along this line. In the work here referred to, 145 Danish soils from varying conditions were examined for Azotobacter, of which 53 per cent gave negative results. The reactions to litmus of 142 of these samples were recorded. The methods used are certainly not free from objections. Of 22 recorded as acid, only one gave Azotobacter. Fifty were recorded as neutral, and of these, 14 per cent gave Azotobacter. Eighty-seven per cent of the 70 recorded as alkaline gave positive cultural results.

The calcium-carbonate content of 136 of the same samples was determined by decomposing with concentrated hydrochloric acid and measuring the carbondioxid evolved. Forty-seven samples contained no calcium carbonate. Christensen states that all samples recorded as 0.05 per cent or less should be regarded as containing no carbonates. Of these 47, 32 per cent gave positive evidence of Azotobacter. Of 102 containing less than 0.10 per cent of calcium carbonate, 33 per cent gave Azotobacter cultures. There were 34 samples giving more than 0.10 per cent of calcium carbonate, and of these, 88 per cent gave Azotobacter, while all of the 23 samples containing more than 0.20 per cent gave positive results. None of the soils recorded as acid or neutral contained sufficient carbonates to replace the calcium carbonate of the cultural solution. Weis and Bornebusch,[2] however, studying the same problem in Danish forest soils found Azotobacter in only 2 out of 64 samples; nevertheless, 60 per cent of these soils contained sufficient carbonates to replace the calcium carbonate of Beijerinck's cultural solution. The last-named authors state that none of the soils examined by them could be regarded as requiring lime.

It would seem from the available experimental data that Azotobacter are capable of existing in many soils which contain none or only traces of calcium carbonate, and also in some soils reacting acid as ordinarily tested. The reaction, however, apparently plays a much more important rôle than the presence of calcium carbonate.

[1] CHRISTENSEN, H. R. STUDIEN ÜBER DEN EINFLUSS DER BODENBESCHAFFENHEIT AUF DAS BAKTERIENLEBEN UND DEN STOFFUMSATZ IM ERDBODEN. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 43, No. 1/7, p. 1–166, 81 fig., 1 pl. Literatur, p. 163–165. 1915.

[2] WEIS, Fr., and BORNEBUSCH, C. H. OM AZOTOBACTERS FOREKOMST I DANSKE SKOVE, SAMT OM AZOTOBACTERPRØVENS BETYDNING FOR BESTEMMELSEN AF SKOVJORDENS KALKTRANG. (Abstract.) *In* Mo. Bul. Agr. Intel. and Plant Diseases, year 6, no. 4, p. 546–548. 1915. (Original article in Forstl. Forsøgsvæsen Danmark, Bd. 4, Haefte 4, p. 319–337. 1914. Not seen.)

Very few, if any, local soils are regarded, agriculturally speaking, as deficient in lime. In fact, the application of lime far in excess of supposed requirements to soils on the Agronomy farm of the Kansas Experiment Station has been without effect upon productivity even when alfalfa was grown. Many of the soils herein reported as containing no Azotobacter were collected from the Agronomy farm. Sample 12 is from a plat to which lime has been applied, yet which failed to show Azotobacter.

Since the writer was unable to associate the presence of Azotobacter with any other factor studied, and since the apparent correlation of their presence with soil reaction was known, this factor was deemed worthy of investigation. A study of the influence of soil reaction seemed especially important, since more exact methods are now available for determining soil acidity.

Recent investigation in other lines of bacteriology have shown that in many instances the degree of acidity or hydrogen-ion concentration is perhaps much more important in controlling bacterial activity than the total or titratable acidity. It has been shown in a number of instances that the degree of acidity tolerated by certain species of bacteria has a very definite limit. Furthermore, none of the methods in vogue for ascertaining the total or titratable acidity of soils are very satisfactory. For these reasons it was thought best, if possible, to determine the reaction in terms of hydrogen-ion concentration. For this purpose the writer has made use of the colorimetric method outlined by Clark and Lubs[1] as recently modified for soils by Gillespie.[2] The indicators used were methyl red, bromcresol purple, bromthymol blue, and phenol red. The standard hydrogen-ion-concentration solutions were prepared as directed by Clark and Lubs, and their accuracy tested and corrected, if need be, by means of electrometric measurements. Little difficulty was experienced in checking with different indicators except in those solutions falling on the acid side of bromcresol purple and the alkaline side of methyl red. Perhaps propyl red would have obviated this difficulty, but none was available when these analyses were made. However, any error arising from this difficulty can in no way vitiate conclusions that may be drawn from these experiments.

In the last column of Table I is given the hydrogen-ion concentration observed in the soil extract, expressed in the usual way—that is, P_H. These results are certainly very striking. Of those soils in which no Azotobacter were observed, all with the exception of three gave a P_H of 5.9 or less. All of the soils which gave Azotobacter growth,

[1] Clark, W. M., and Lubs, H. A. The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. *In* Jour. Bact., v. 2, no. 1, p. 1–34; no. 2, p. 109–136, no. 3, p. 191–236, 8 fig. 1917. References, p. 233–236.

[2] Gillespie, L. J. The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, p. 7–16, 2 fig. 1916.

except three, gave a P_H of 6.0 or above. The average P_H of soils showing no Azotobacter growth was 5.71 and the nitrogen fixed 3.88 mgm. per culture. The average P_H of those soils showing Azotobacter growth was 6.78 and the average nitrogen fixed was 8.11 mgm. per culture. Of the exceptions to the above rule, soil 38 gave very few isolated colonies, and in the case of No. 76 only one culture gave Azotobacter. In these two instances the Azotobacter growth was probably due to contamination. All these exceptions are being studied further.

It should be remembered that the acidity analyses were made on samples of soil that had been stored from 7 to 10 months. Gillespie has called attention to the possibility of determinations made under such conditions varying slightly from actual soil conditions.

The writer does not, therefore, wish to leave the impression that the the maximum acidity tolerated by Azotobacter is necessarily represented exactly by a P_H of 6.0, or that the limits are necessarily so definite as these experiments would indicate. It is believed, however, that the results herein reported are very significant. Also that when investigations now under way are completed, the writer will be in a position to say that active Azotobacter will not exist in soils in which, other factors not interfering, the hydrogen-ion concentration exceeds a fairly definite limit He hopes also to give much more accurate data as to what that limit is. The same phenomenaare being studied in the case of other soil organisms.

EFFECT OF DIFFERENT OXYGEN PRESSURES ON THE CARBOHYDRATE METABOLISM OF THE SWEET POTATO

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INTRODUCTION

The recognizable products which are formed as a result of starch transformation in the sweet potato (*Ipomoea batatas*) during storage are reducing sugars and cane sugar. According to Miyaki [1] the reducing sugars consist of glucose and possibly fructose. Maltose has not been found. The main product is cane sugar, which has been frequently identified.

In the course of ordinary storage the monosaccharids soon reach their maximum concentration, which in Big Stem and Southern Queen rarely exceeds 2 per cent of the weight of the fresh potato. The cane sugar continues to accumulate until in the varieties named it represents as much as 7 per cent of the fresh potato.[2]

The fact that the reducing sugars remain at a low concentration while the cane sugar continues to accumulate suggested that the reducing sugar is an intermediate product in the transformation of starch to cane sugar in the sweet potato in storage. Evidence that the changes proceed in this manner was obtained by a study of the process at low temperatures by which the rates of the different steps in the series of changes are unequally modified.[3] It was thus shown that the production of reducing sugar antecedes the formation of cane sugar. That a further separation of the various steps in this transformation, or possibly a suppression of one or more of the phases of the process, could be brought about by other means, such as changes in oxygen pressure, seemed not improbable, especially since Cruickshank in 1797 [4] had observed that soaked barley seeds failed to become sweet in the absence of oxygen, and Boysen-Jensen [5] more recently found that cane sugar was not formed

[1] MIYAKI, K. ON THE NATURE OF THE SUGARS FOUND IN THE TUBERS OF SWEET POTATOES. *In* Jour. Biol. Chem., v. 21, no. 2, p. 503-506. 1915.

[2] HASSELBRING, Heinrich, and HAWKINS, L. A. PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE. *In* Jour. Agr. Research, v. 3, no. 4, p. 331-342. 1915. Literature cited, p. 341-342.

[3] HASSELBRING, Heinrich, and HAWKINS, L. A. CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES. *In* Jour. Agr. Research, v. 5, no. 13, p. 543-560. 1915.

[4] CRUICKSHANK, William. SOME EXPERIMENTS AND OBSERVATIONS ON THE NATURE OF SUGAR. *In* Rollo, John. An account of two cases of the diabetes mellitus . . . v. 2, p. 210-226. London, 1797. Reprinted in Jour. Nat. Phil., Chem., and Arts [Nicholson], v. 1, p. 337-341. 1797. French trans. by Guyton in Ann. Chim., v. 25, p. 37-50. 1798.

[5] BOYSEN-JENSEN, P. ÜBER SYNTHETISCHE VORGÄNGE IM PFLANZLICHEN ORGANISMUS. I. DIE ROHRZUCKERSYNTHESE. *In* Biochem. Ztschr., Bd. 40, Heft 5/6, p. 420-440, 2 fig. 1912.

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in germinating barley and peas under similar conditions. With this idea in view a study of the effects of different oxygen pressures on the carbohydrate transformation in the sweet potato was undertaken.

EXPERIMENTAL METHODS

The general method adopted was that heretofore used of cutting sweet potatoes into halves lengthwise and storing one half under experimental conditions, while the other half was prepared for immediate analysis. The potatoes were always dug in the afternoon, properly cleaned, and stored in a cool place until the following day, when they were prepared for the experiments. Either five or six individuals were used for each experiment.

With respect to the total gas pressure to which the stored halves were subjected, the experiments may be divided into three groups: (1) experiments at pressures greater than one atmosphere; (2) experiments at atmospheric pressure; and (3) one experiment at a pressure of less than one atmosphere.

In the experiments at pressures greater than one atmosphere the potatoes were stored in a gas-tight iron cylinder 33 cm. high and 22.9 cm. in diameter. The pressures were measured by means of a standard test gauge in the head of the cylinder. The whole apparatus, except the projecting gauge, was submerged in a constant temperature water bath. Preliminary experiments showed that with an inside pressure of 10 atmospheres there was no leakage from the cylinder during a period of 10 days.

In the second group of experiments the potatoes were stored in oxygen, air, or hydrogen at atmospheric pressure. For this purpose desiccators kept in constant temperature chambers were used. For the experiments in air the desiccators were merely ventilated, but in the other experiments the gases were passed through the desiccators in a rapid stream until all the air had been replaced. Thereupon the current was slowed down until 60 to 100 bubbles of gas per minute passed through wash bottles at the exits. Before entering the desiccators the gases passed through about 10 meters of copper tubing coiled in the constant temperature chambers and then through wash bottles filled with water. Short thermometers placed in these wash bottles showed that the gases entered the desiccators at the temperature of the chambers. The oxygen and hydrogen were obtained from cylinders furnished by a commercial company. The hydrogen contained approximately 0.17 to 0.25 per cent of oxygen. In two experiments, as will be mentioned later, these small traces of oxygen were removed from the hydrogen.

The single experiment in which the potatoes were stored at less than atmospheric pressure was carried out by means of the gas cylinder described above.

EXPERIMENTAL WORK

EXPERIMENTS AT PRESSURES GREATER THAN ONE ATMOSPHERE

Two experiments were conducted under pressures greater than one atmosphere. These require only a brief discussion, since in both cases the sweet potatoes were killed. In the first experiment the potatoes were stored for 10 days in air at 30° C. and under a pressure of 10 atmospheres. At the end of that period the potatoes showed a few spots where organisms had developed; otherwise the tissues were intact but killed. No analyses of these were made. In the second experiment the potatoes were kept for 5 days in oxygen at 30° and at a pressure of 5 atmospheres. These also were killed. The tissues were watery but firm and, so far as could be observed microscopically, were free from fungi and bacteria. Two of the halves were analyzed. The results are given in Table I. In this and in the subsequent tables the halves analyzed at the beginning of the experiment are marked "a," and the stored halves are marked "b."

TABLE I.—*Changes in composition of sweet potatoes stored for 5 days in oxygen at 30° C. and at a pressure of 5 atmospheres*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1a	75.50	17.09	0.31	1.63	69.76	1.27	6.65
1b	73.85	17.78	1.75	.63	67.99	6.69	2.41
6a	71.14	21.39	.40	1.72	74.12	1.39	5.96
6b	71.04	20.70	1.81	.95	71.48	6.25	3.28

The data show that under these conditions the hydrolysis of starch proceeded to a very limited extent, and that not only did no synthesis of cane sugar take place but cane sugar disappeared. As a result of the hydrolysis of starch and cane sugar there was a considerable accumulation of reducing sugar. To what extent these changes took place in the living potato it is not possible to say. It is likely from the disappearance of cane sugar that the roots were quickly killed and that the synthesis of cane sugar at least does not go on in killed tissues. The hydrolysis of starch also is greatly retarded. Since the whole problem of the effects of high pressures on the metabolism of the plant organs requires a detailed investigation, no further experiments in this field were conducted at this time.

EXPERIMENTS AT ATMOSPHERIC PRESSURE

In the first set of experiments at atmospheric pressure different lots of halved sweet potatoes were stored for five days in oxygen, air, and

hydrogen, respectively, at a temperature of 30° C. The changes in composition of the stored halves as compared with the halves analyzed immediately are given in Table II.

TABLE II.—*Changes in composition of sweet potatoes stored for five days under different conditions*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED IN OXYGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
7a	77.07	15.16	0.21	1.82	66.11	0.92	7.94
7b	76.96	11.92	1.10	3.97	51.74	4.77	17.23
8a	75.96	15.87	.23	2.10	66.02	.96	8.74
8b	76.65	13.23	.98	3.27	56.66	4.20	14.00
9a	76.10	15.80	.30	2.08	66.12	1.26	8.70
9b	74.87	13.74	1.34	3.81	54.68	5.33	15.16
10a	76.70	15.75	.43	1.94	67.60	1.84	8.33
10b	77.08	12.57	1.69	2.98	54.84	7.37	13.00
11a	76.01	16.19	.25	1.95	67.49	1.04	8.13
11b	73.72	15.67	1.23	3.35	57.34	4.68	12.75
12a	76.32	15.79	.32	2.13	66.68	1.35	9.00
12b	77.65	11.89	1.39	3.52	53.20	6.22	15.75
STORED IN AIR AT 30° C. AND AT ATMOSPHERIC PRESSURE							
13a	76.28	15.92	0.29	2.09	67.12	1.22	8.81
13b	76.35	14.05	1.45	3.21	59.41	6.13	13.57
14a	75.78	16.18	.30	2.19	66.81	1.24	9.04
14b	75.87	14.97	1.32	2.10	62.04	5.47	8.70
15a	77.54	14.69	.25	2.11	65.40	1.11	9.39
15b	77.04	13.70	1.15	2.67	59.67	5.01	11.63
16a	76.46	16.06	.41	1.75	68.22	1.74	7.43
16b	75.90	15.03	1.46	2.31	62.37	6.06	9.59
17a	75.92	16.80	.25	2.11	67.26	1.00	8.45
17b	74.95	15.18	.77	3.11	60.60	3.07	12.42
18a	76.08	15.78	.25	2.12	65.97	1.05	8.86
18b	74.75	15.03	.85	3.16	59.53	3.37	12.52
STORED IN HYDROGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
19a	76.47	15.74	0.18	1.78	66.89	0.77	7.56
19b	77.26	11.94	.29	4.36	52.51	1.28	19.17
20a	77.15	13.57	.23	1.84	68.14	1.01	8.05
20b	77.47	12.44	.28	4.15	55.22	1.24	18.42
21a	78.08	14.63	.17	1.93	66.75	.76	8.80
21b	78.18	11.75	.34	4.50	53.85	1.56	20.62
22a	76.83	15.11	.16	2.30	65.21	.69	9.93
22b	77.50	11.69	.26	4.78	51.96	1.16	21.25
23a	76.86	15.63	.18	2.03	67.55	.78	8.77
23b	77.16	12.57	.32	4.50	55.04	1.40	19.70
24a	77.60	15.30	.41	1.61	68.30	1.83	7.19
24b	79.01	10.93	.41	4.22	52.07	1.95	20.10

At the end of the experiment the stored halves were fresh and crisp and in perfect condition. The only difference noted between those stored in air or oxygen and those stored in hydrogen was that in the roots stored in air or oxygen the oxidizable chromogenic material exuding from the cut surfaces was darkened, while in those stored in hydrogen discoloration was entirely absent, but appeared as soon as the potatoes were exposed to the air.

These data show that the formation of cane sugar[1] in the sweet potato is not inhibited nor depressed in an atmosphere practically free from oxygen. In regard to the quantitative effects of the different oxygen pressures it seems clear that both in an atmosphere of oxygen and in an atmosphere practically free from oxygen more starch disappears and more cane sugar is formed than in air. The percentage of cane sugar is greatest in the potatoes stored in hydrogen.

Since cane sugar is apparently not utilized in respiration by the sweet potato, the loss of other materials through respiration would result in an increase of the percentage of cane sugar without an increase in the actual quantity present. However, the possible difference in loss of material by respiration in air and in hydrogen is not sufficiently great to account for the greater percentage of cane sugar in the potatoes stored in hydrogen. It appears, therefore, that in the absence of oxygen cane sugar is actually produced more rapidly than in air. A further fact worthy of note is that at the temperature of these experiments there is practically no increase in reducing sugar in the potatoes stored in hydrogen.

[1] That the nonreducing sugar formed in the sweet potato in the absence of oxygen is cane sugar was shown by the following experiments:

Four small sweet potatoes, weighing together 1075.5 gm., were cut into halves. One lot of halves weighing 555.5 gm. was grated immediately. From the mash three 25-gm. samples were taken for sugar determinations. The remaining mash, after the removal of these samples, weighed 475 gm., some loss having resulted from the adherence of material to the grater. From this mash the sugar was quantitatively extracted with 70 per cent alcohol, first by repeated decantation in the cold, and finally by means of a Soxhlet apparatus. After concentration of the extract under reduced pressure the sugar was isolated as barium saccharate, from which it was recovered in crystallized form. The quantity of nonreducing sugar present in the mash, according to determination, was 14.9 gm. The sugar recovered from the barium saccharate, after having been washed with glacial acetic acid and alcohol, weighed 13.22 gm.; 0.6014 gm. dissolved and made up to 50 cc. at 20°C., gave an angular rotation in a 4-dcm. tube of 3.169°, to the right equivalent to a specific rotation of +65.9°. The remainder of the sugar was recrystallized from alcohol and yielded 12.05 gm.; 0.7440 gm. dissolved and made up to 50 cc. as before gave an angular rotation of 3.953°, or a specific rotation of +66.4°.

The second lot of halves, weighing 519.5 gm., was stored for 15 days in the manner described in the text in an atmosphere of hydrogen entirely freed from oxygen. At the end of that period these halves, whose weight had decreased to 505.0 gm., were treated like the first lot. The mash, after removal of the samples for sugar determination weighed 421 gm., and according to determination contained 24.42 gm. of nonreducing sugar. The quantity theoretically present in the equivalent of the mash

before the experiment was 13.60 gm., correction having been made for the loss of weight of the halves during the experiment. The yield from the first crystallization was 22.47 gm., 0.7357 gm. dissolved and made up to 50 cc. at 20°C. gave an angular rotation in a 4-dcm. tube of 3.886° to the right, or a specific rotation of +66°. The residue on recrystallization yielded 20.69 gm.; 0.5450 gm. dissolved as before gave an angular rotation of 2.896°, equivalent to a specific rotation of +66.4°. The specific rotation of cane sugar is 66.5°. The quantity of sugar recovered from the last recrystallization is 7.09 gm. in excess of the quantity present in an equivalent of the mash before the experiment.

In a second experiment a single large sweet potato weighing 884.5 gm. was used. The potato was cut lengthwise and the halves treated as described above, with the exception that the mash was extracted first by decantation and finally by percolation in the cold. The stored half, weighing 471 gm., lost 12 gm. during the experiment. From the mash of the first half, 9.25 gm. of recrystallized sugar were obtained; 4.4860 gm. of this dissolved and made up to 50 cc at 20° C. gave an angular rotation in a 2 dcm. tube of 11.972°, or a specific rotation of +66.7°. The yield of recrystallized sugar from the stored half was 19.02 gm.; 4.1003 gm. dissolved as before gave an angular rotation of 10.892°, or a specific rotation of +66.4°. The quantity of nonreducing sugar originally present in the equivalent of this mash, according to determination and after correction for the loss of weight during storage, was 13.31 gm. The recrystallized sugar therefore represents an increase of 5.71 gm.

The complete data from these experiments are tabulated here.

	First experiment.		Second experiment.	
	I.	II. (Stored half).	I.	II. (Stored half).
Weight of halves.....gm..	555.5	519.5	413	471
Weight of stored half at end of experiment.....gm..	.....	505	.....	459
Weight of mash extracted.....gm..	475	421	332	376
Weight of sucrose present according to determination.....gm..	14.91	24.42	11.45	22.37
Sucrose in mash of II at beginning of experiment.....gm..	.....	13.60	.....	13.31
Yield of sugar from first crystallization.gm..	13.23	22.47	10.67	21.50
Specific rotation at 20°C.....°	+65.9°	+66.0°	.....	.....
Yield of sugar after one recrystallization.gm..	12.05	20.69	9.25	19.02
Specific rotation at 20°C.....°	+66.4	+66.4	+66.7	+66.4

The specific rotation of the recrystallized product obtained in these experiments identifies as cane sugar the non-reducing sugar present in the freshly dug sweet potato as well as the additional non-reducing sugar formed during storage in the absence of oxygen. To Dr. C. S. Hudson, of the Bureau of Chemistry, the writer is indebted for helpful suggestions regarding the isolation and identification of the sugar from the sweet potato.

A second series of experiments was carried out under the same conditions but the halves were kept in the desiccators for 10 days. At the end of that period the potatoes were in perfect condition, as in the first experiments. The results of this series are given in Table III.

The statements made in regard to the first set of experiments apply equally well to this set. In general the change in the 10-day period is scarcely greater than that during the 5-day period. Only in the potatoes

stored in air is a noticeable further increase of sugar apparent. In oxygen and in hydrogen a state of equilibrium is practically reached in five days. In these experiments also there is only a slight increase in reducing sugar in the absence of oxygen.

TABLE III.—*Changes in composition of sweet potatoes stored for 10 days under different conditions*

Sweet potato. No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED IN OXYGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
25a	77.31	15.31	0.31	1.92	67.48	1.37	8.46
25b	76.66	13.57	1.68	2.68	58.14	7.20	11.48
26a	75.94	16.45	.22	1.71	68.37	.91	7.11
26b	76.88	12.81	1.20	3.32	55.41	5.19	14.36
27a	75.77	16.63	.32	2.12	68.64	1.32	8.75
27b	76.14	13.77	1.44	3.21	57.71	6.04	13.46
28a	73.85	18.76	.23	1.94	71.74	.88	7.42
28b	75.07	14.63	1.33	3.64	58.69	5.34	14.60
29a	72.82	19.81	.42	1.90	72.88	1.55	6.99
29b	72.40	17.37	1.37	3.09	62.94	4.96	11.20
30a	71.94	20.70	.34	1.93	73.77	1.21	6.88
30b	71.96	17.42	1.42	3.40	62.13	5.06	12.13
STORED IN AIR AT 30° C. AND AT ATMOSPHERIC PRESSURE							
31a	75.86	15.90	0.30	2.55	65.87	1.24	10.56
31b	74.63	15.58	1.36	3.16	61.41	5.37	12.46
32a	73.47	18.41	.23	2.38	69.39	.87	8.71
32b	72.72	17.19	.79	3.45	63.01	2.89	12.65
33a	73.97	18.04	.27	2.40	69.31	1.04	9.22
33b	73.97	15.96	.83	3.57	61.32	3.19	13.72
34a	76.14	15.97	.24	2.13	66.93	1.00	8.93
34b	75.40	14.49	1.06	3.56	58.90	4.31	14.47
35a	76.33	16.24	.45	2.05	68.61	1.90	8.66
35b	75.26	14.50	1.17	3.63	58.61	4.73	14.67
36a	74.59	17.55	.36	2.48	69.07	1.42	9.76
36b	73.80	15.93	1.07	3.70	60.80	4.08	14.12
STORED IN HYDROGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
37a	75.10	17.42	0.27	2.00	69.96	1.08	8.03
37b	77.21	12.56	.51	4.26	55.11	2.24	18.69
38a	75.73	16.21	.18	2.43	66.79	.74	10.01
38b	77.07	12.27	.28	4.51	53.51	1.22	19.67
39a	74.70	17.33	.17	2.22	68.50	.67	8.77
39b	75.89	13.34	.31	4.48	55.33	1.29	18.58
40a	74.55	17.59	.22	2.37	69.12	.86	9.31
40b	76.24	13.50	.29	4.39	56.82	1.22	18.48
41a	75.80	16.26	.28	2.36	67.19	1.16	9.75
41b	75.84	13.75	.27	4.64	56.91	1.12	19.21
42a	74.50	18.21	.27	1.87	71.41	1.06	7.33
42b	75.64	14.43	.37	4.03	59.24	1.52	16.54

In experiments reported in previous papers it was found that an increase in reducing sugar precedes or accompanies the increase in cane sugar in the sweet potato. From these observations the conclusion was drawn that the monosaccharides result from the hydrolysis of starch, and that cane sugar is synthesized from these. The failure of reducing sugar to accumulate in an atmosphere containing only traces of oxygen might be attributed to two causes. First, to its more rapid utilization in the formation of cane sugar, and, second, to a greater demand for materials to sustain anaerobic respiration at the temperature at which the experiments were conducted. If, therefore, these processes, especially the respiration, could be retarded without retarding in a corresponding degree the hydrolysis of starch reducing sugar ought to accumulate in the absence of oxygen in the same manner as in air. To settle this point, a series of four experiments was carried out in which the potatoes were stored for different lengths of time from 3 to 20 days in hydrogen at a temperature of 4.5° C.

Since, as has been stated, the hydrogen used in these experiments contained traces of oxygen and as no tendency toward the suppression of the formation of cane sugar was evident, it might be urged that the small traces of oxygen mixed with the hydrogen from the cylinders were sufficient to stimulate the processes leading to the formation of cane sugar. It therefore became necessary to exclude these traces in order to determine definitely whether cane sugar could be formed in the sweet potato in the absence of all traces of oxygen. For this purpose the hydrogen used in the last two experiments (Table IV, 10 and 20 days) was passed through a tube containing heated palladium asbestos. Analyses over mercury of the gas issuing from the chambers made on the day after the experiments were set up showed no oxygen present.

The results of this series of experiments are given in Table IV.

Table IV shows clearly the course of the carbohydrate changes in the absence of oxygen. After three days almost no change has taken place. After five days an increase in reducing sugar becomes apparent, but the increase in cane sugar is very slight. At the end of 10 days the reducing sugar has increased to from two to four times the original quantity, while the cane sugar still shows but very little increase. During the next 10 days there is a further increase in reducing sugar, but this period is characterized mostly by the great increase in cane sugar.

These facts show that the carbohydrate transformations in the sweet potato proceed in the same manner under anaerobic conditions as they do under aerobic conditions. The failure of reducing sugar to accumulate at high temperatures under anaerobic conditions is probably in part attributable to its more extensive utilization in respiration.

The data in the last two experiments (Table IV, 10 and 20 days) show that even in the entire absence of oxygen the formation of cane sugar is possible in the sweet potato. It is evident, therefore, that no significance need be attributed to the effects of the small traces of oxygen contained in the hydrogen used in the other experiments.

TABLE IV.—*Changes in composition of sweet potatoes stored in hydrogen at 4.5° C. and at atmospheric pressure for different periods*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED FOR 3 DAYS							
43a	73.38	19.00	0.32	1.96	71.37	1.20	7.36
43b	73.72	18.81	.43	2.15	71.58	1.64	8.18
44a	75.76	16.90	.43	2.11	69.72	1.77	8.70
44b	75.15	17.39	.53	2.16	69.62	2.13	8.69
45a	73.70	18.88	.30	2.04	71.79	1.14	7.76
45b	72.07	20.27	.23	2.18	72.57	.82	7.81
46a	73.56	19.04	.47	2.16	72.01	1.78	8.17
46b	73.74	18.77	.65	2.34	71.48	2.48	8.91
47a	73.21	19.31	.35	2.05	72.08	1.31	7.65
47b	72.96	19.49	.41	2.10	72.08	1.52	7.77
STORED FOR 5 DAYS							
48a	73.34	19.07	0.35	2.30	71.53	1.31	8.63
48b	75.10	16.93	.66	2.31	67.99	2.65	9.28
49a	72.35	20.18	.24	1.97	72.98	.87	7.12
49b	73.10	18.88	.47	2.21	70.19	1.75	8.22
50a	73.99	18.79	.31	1.77	72.24	1.19	6.81
50b	73.65	18.50	.58	1.94	70.21	2.20	7.36
51a	71.44	21.28	.38	1.92	74.51	1.33	6.72
51b	73.27	18.70	.89	2.08	69.96	3.33	7.78
52a	75.09	17.61	.35	2.07	70.70	1.41	8.31
52b	74.30	17.76	.56	2.21	69.11	2.18	8.60
STORED FOR 10 DAYS							
53a	74.58	17.55	0.38	2.07	69.04	1.49	8.14
53b	76.16	15.73	1.48	1.92	65.98	6.21	8.08
54a	74.76	17.78	.41	2.34	70.44	1.62	9.27
54b	75.30	16.47	1.39	2.29	66.68	5.63	9.27
55a	73.28	18.99	.45	2.66	71.07	1.68	9.96
55b	74.06	17.66	1.08	2.94	68.08	4.16	11.33
56a	71.07	21.58	.42	2.23	74.60	1.45	7.71
56b	72.15	19.96	1.03	2.59	71.67	3.70	9.30
57a	75.64	16.90	.58	2.18	69.38	2.38	8.95
57b	76.73	15.05	1.69	1.94	64.68	7.26	8.34
STORED FOR 20 DAYS							
58a	76.34	16.52	0.59	1.98	69.82	2.49	8.37
58b	77.60	13.22	1.51	2.87	59.02	6.74	12.81
59a	76.37	16.43	.82	2.02	69.53	3.47	8.55
59b	77.88	12.93	1.76	2.91	58.46	7.96	13.15
60a	76.57	16.40	.70	1.80	70.00	2.99	7.68
60b	78.34	12.94	1.40	2.92	59.74	6.46	13.48
61a	76.52	16.17	.59	1.89	68.87	2.51	8.05
61b	77.05	13.32	1.43	3.30	58.04	6.23	14.38
62a	75.68	16.78	.63	2.17	69.00	2.59	8.92
62b	77.56	12.79	1.67	3.29	57.00	7.44	14.66

EXPERIMENT UNDER A PRESSURE OF LESS THAN ONE ATMOSPHERE

A single experiment was carried out in which the sweet potatoes were stored in a vacuum chamber which was kept in a water bath at 30° C. For this purpose the gas cylinder described above was used. The air was exhausted to a pressure of 4 mm. A dish of moist soda-lime was placed on the bottom of the chamber to absorb the carbon dioxid. The potatoes were kept in the chamber for five days. Owing to the absorption of water by the soda-lime the interior of the chamber was very dry and the potatoes were much wilted, but otherwise uninjured. The results of this experiment are given in Table V.

TABLE V.—*Changes in composition of sweet potatoes stored for 5 days at 30° C. and at a pressure of 4 mm.*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
63a	76.74	15.51	0.35	2.27	66.68	1.54	9.76
63b	76.78	10.29	.55	6.06	44.32	2.37	26.10
64a	76.88	15.27	.32	2.30	66.05	1.38	9.95
64b	76.80	10.61	.47	5.87	45.73	2.03	25.30
65a	78.19	13.97	.50	2.22	64.05	2.29	10.18
65b	76.29	10.18	.91	6.11	42.94	3.84	25.77
66a	77.59	14.59	.29	2.45	65.10	1.29	10.93
66b	70.87	14.83	.31	6.45	50.91	1.06	22.14
67a	75.01	16.87	.24	2.29	67.51	.96	9.16
67b	73.86	12.09	.38	6.44	46.25	1.45	24.64
68a	77.66	14.40	.43	2.33	64.46	1.92	10.43
68b	77.94	10.15	.61	5.43	46.01	2.77	24.61

It is very probable that the available oxygen within the cylinder was soon removed by the potatoes and that thereafter they were in an atmosphere free from oxygen. The behavior of the potatoes under these conditions is like that of the potatoes stored in hydrogen at the same temperature. There is a marked accumulation of cane sugar but scarcely any increase in reducing sugar.

DISCUSSION OF RESULTS

In the experiments of Cruickshank the lack of sweet taste in the soaked barley kept in an atmosphere free from oxygen may be taken to indicate the absence not only of cane sugar, which, according to O'Sullivan,[1] may constitute as much as 4.5 per cent of the dry weight of the germinated grain, but also of the other sugars occurring in malt. It is therefore reasonably sure that under the conditions of the experiments no cane sugar was formed. In like manner the experiments of Boysen-Jensen show that in

[1] O'SULLIVAN, C. ON THE SUGARS OF SOME OF THE CEREALS AND OF GERMINATED GRAIN. (Abstract.) In Chem. News, v. 52, no. 1359, p. 293. 1885.

the absence of oxygen cane sugar is not formed in germinating peas and barley. Both of these investigators conclude that the presence of oxygen is one of the necessary conditions for the formation of cane sugar. This conclusion, however, is not of general validity, since the experiments with sweet potatoes show that cane sugar can be formed in some plant organs in the absence of oxygen. Possibly this difference in behavior of sweet potato roots and germinating seeds is associated with the difference in the degree of activity between dormant organs and active embryos or seedlings.

From the correlation which he observed between the formation of cane sugar and aerobic respiration Boysen-Jensen believes that the respiratory process furnishes the energy necessary for the synthesis of cane sugar. If such a relation between respiratory energy and sugar synthesis exists it is not surprising that in some cases, as in the sweet potato, the requisite energy can be derived also from the processes of anaerobic respiration. In such cases, however, we should expect to find the quantity of material consumed in anaerobic respiration greater than that consumed in normal respiration, since the energy derived from a given mass of material is not equal in the two cases.

Two lots of halved sweet potatoes weighing, respectively, 1,273 and 1,479 gm., were placed in respiration chambers at 30° C. Through the first chamber a rapid current of air was passed for three days and through the second a current of hydrogen was passed in the same manner. The daily carbon-dioxid output in grams per kilogram of the two lots of roots for the next five days was as follows:

LOT I: POTATOES IN AIR.	LOT II: POTATOES IN HYDROGEN.
1.44	1.67
1.32	1.81
1.38	1.85
1.12	2.03
1.01	2.32

On the hypothesis that in normal respiration glucose is completely oxidized to carbon dioxid and water, while in anaerobic respiration carbon dioxid and alcohol are formed, 1 gm. of carbon dioxid in normal respiration is equivalent to 0.682 gm. of glucose and in anaerobic respiration to 2.045 gm. of glucose. It is seen, therefore, that the quantity of material consumed in anaerobic respiration is actually much greater than that consumed in normal respiration. Moreover, in normal respiration the quantity of material consumed decreases as the plant adjusts itself to the conditions, while in anaerobic respiration the quantity increases. In a general way, therefore, the experiments reported in this paper and those reported in former papers seem to give some support to Boysen-Jensen's theory in so far as the production of cane sugar is greatest under conditions of greatest utilization of material by respiration.

CONCLUSIONS

Under gas pressure of 5 atmospheres or more sweet potatoes are killed. In the killed tissues starch hydrolysis is greatly depressed or inhibited. Cane sugar is converted by hydrolysis into reducing sugars which accumulate.

Starch hydrolysis and cane sugar formation in the sweet potato proceed in the absence of oxygen in the same manner as in air or in an atmosphere of oxygen. The presence of oxygen is therefore not always a necessary condition for the formation of cane sugar in plant organs.

The quantity of material consumed in a given period of time in anaerobic respiration by the sweet potato is greater than the quantity consumed in normal respiration at the same temperature. The actual carbon-dioxid output is also greater under anaerobic conditions. Cane sugar appears not to be consumed in either process.